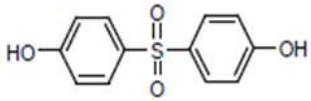
NTP
National Toxicology Program

Bisphenol S

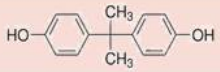
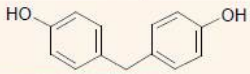
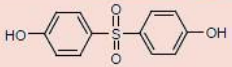
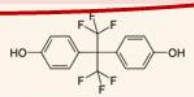
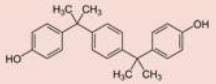
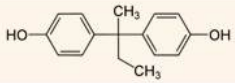


Vicki Sutherland, PhD
National Institute of Environmental Health Sciences

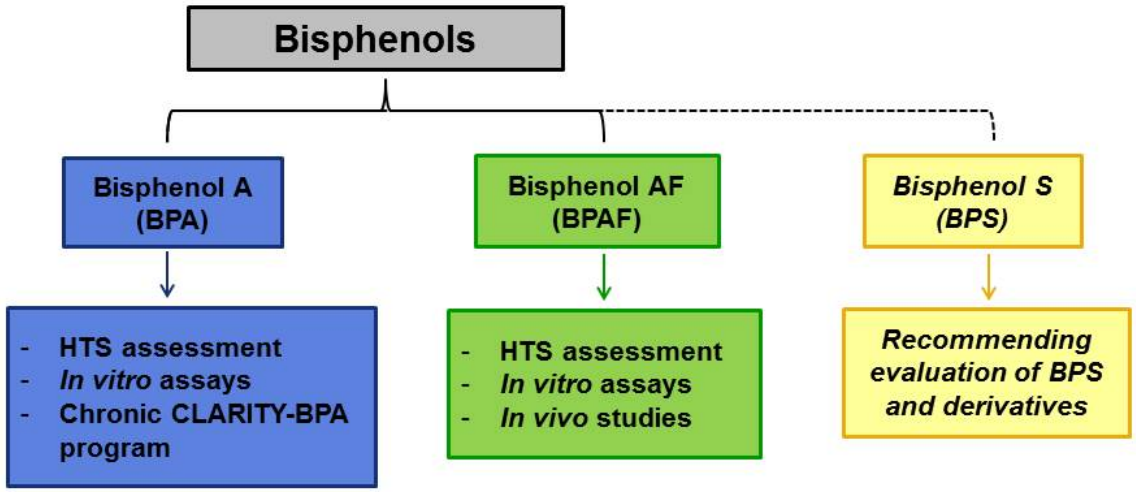
NTP Board of Scientific Counselors Meeting
June 17 - 18, 2014

Bisphenol Analogs

BPA Analogues – structurally similar chemicals.

Example Bisphenol Analogues	Analogue Structures
BPA	
BPF	
BPS	
BPAF	
BPP	
BPB	

Current NTP Toxicological Assessments:



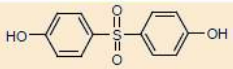
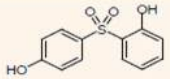
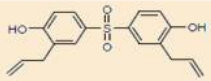
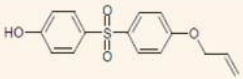
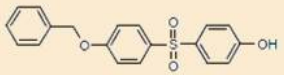
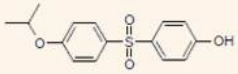
BPA and BPAF evaluations are ongoing – preliminary data indicates that *in vivo* responses between the 2 chemicals are different

Bisphenol S (BPS) and Derivatives:

- Nominated by the EPA and NIEHS:
  - Potential endocrine activity
  - Limited toxicological testing
  - High probability for human exposure
    - Increase in production - changes from BPA to BPS (or derivatives).
      - Produced and/or manufactured in the US and China.
      - Aggregated production volume for BPS:
        - >1 to 10 million pounds from 1986 to 2001
        - 1 to <10 million pounds in 2012 (U.S. EPA 2013)

Bisphenol S Derivatives:

Derivatives – same core structure as BPS.

Example BPS Derivatives	Derivative Structures
BPS	
2,4-BPS 2,4'-Bis(hydroxyphenyl) sulfone	
TGSA Bis-(3-allyl-4-hydroxyphenyl) sulfone	
BPS-MAE Phenol,4-[[4-(2- propen-1-yloxy)phenyl] sulfonyl]	
BPS-MPE 4-Hydroxy-4'-benzyloxydiphenylsulfone	
D-8 4-Hydroxyphenyl 4-isoprooxyphenylsulfone	
D-90 4-[4'-[(1'-methylethyloxy) phenyl]sulfonyl]phenol	

BPS and Derivative Uses:

- Bisphenol S (BPS) is used as a raw material & to produce PES (polyether sulfone) and other polymers, as an alternative to polycarbonate.

  PES = **BPS** + DCDPS (4,4'-dichlorodiphenylsulfone)

- BPS (including BPS derivatives) is of concern because of use of PES and BPS alone in a variety of consumer products and applications:

PES

-Modifier for materials & epoxy resins	-Dialyzer	-Artificial organs & joints
-Binder for non-stick coatings	-Bottle top filter units	-Microwave dishes
-Print cartridges	-Milking Machines	-Baby bottles

BPS

-Developers for heat sensitive paper	-Fire retardants	-Couplers for photography
-Electroplating chemicals	-Intermediate for colorants, pharmaceuticals, pesticides	

Potential routes of exposure:

- Oral: BPS migrates from the lining of plastic products or through consumption of exposed food products
- Dermal: Contact with BPS-containing products (e.g. thermal paper - levels of BPS in cashiers are higher than the general population)
- Inhalation: PES sprayings in an occupational setting
- Blood, lymph, tissues: Through biomedical applications (ie. artificial joints, organs, hemodialyzers, etc.)

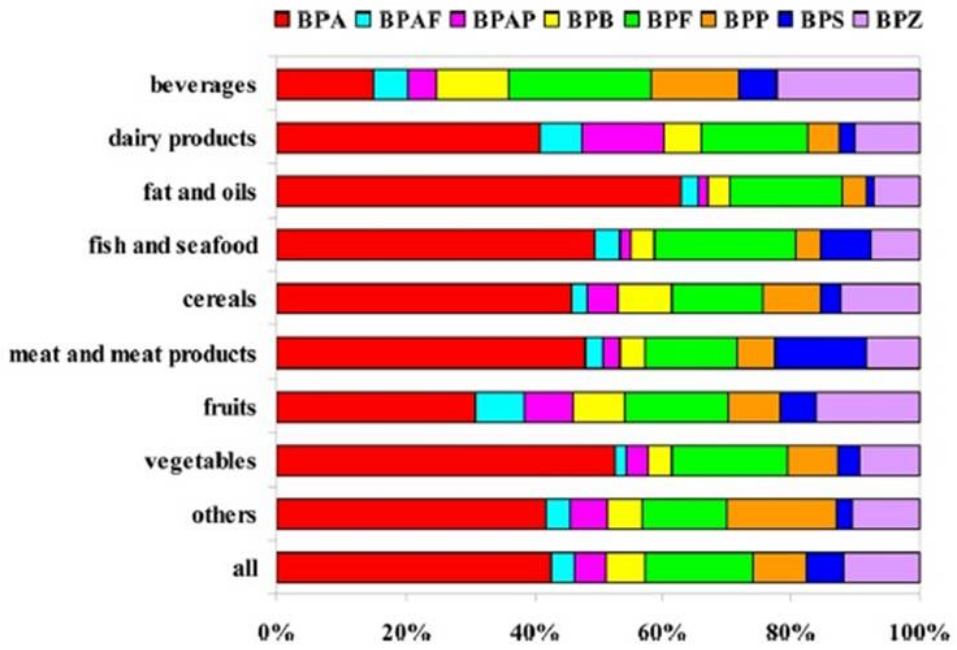
BPS Occurrence:

- BPS, and other Bisphenols, are found:
  - Food (i.e. cereals, seafood, dairy, vegetables, canned products)
  - Indoor dust
  - Sediment (moderately persistent in sediment and has the potential for accumulation in the aquatic environment)
  - Paper and paper products (i.e. toilet paper, cashier's receipts, currency)

❖ Analysis of BPS in human urine samples - 81% of the samples analyzed contained BPS (0.654 ng/mL)

Liao, C., et al., EST 2012 **46:**6860

Bisphenol Analogues in Foodstuffs (Albany, NY)



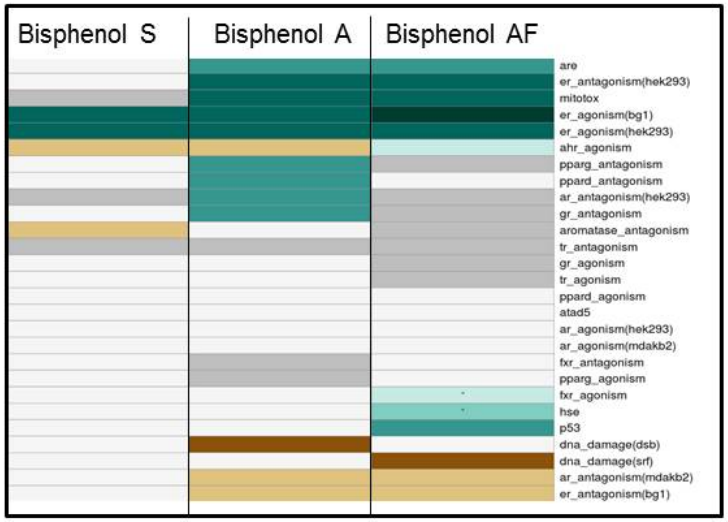
Liao, C. and K. Kannan, Journal of agricultural and food chemistry, 2013. **61:**4655-62.

BPS *In Silico* Information:

- Quantitative structure-activity relationship (QSAR) analyses for BPS:
  - Models predicted carcinogenic, hepatotoxic, nephrotoxic, anemia, and neurotoxic effects
  - Predicted molecular targets:
    - inhibition of MMP-13
    - stromelysin 1
    - transthyretin
    - estrogen receptors

Tox21 High Throughput Screening data and *In Vitro* Information:

- BPS is an estrogen agonist and is active in 2 estrogen receptor assays:



- BPS is an antagonist of or produced no effects on the androgen and thyroid systems (conflicting literature)

BPS *in vivo* study data*:

Study	Results
28-day study in Rats	• Intestinal hemorrhaging and death • Increases in liver, thymus, adrenal and kidney weights and changes in clinical pathology parameters
13-day study in Rats	• Decreased body and organ (liver and kidney) weights • Changes in clinical pathology parameters
Rat Reproductive Toxicology Screening study	• Increased liver weights • Prolongation of the estrous cycle, increases in # irregular cycles, and decreases in fertility index, # implantations, live births, and live offspring at lactation day 4

*Full study report unavailable (Robust Summary Data from REACH Dossiers in the European Chemicals Agency (ECHA) database)

Knowledge Gaps:

- Limited developmental, reproductive, or endocrine evaluations
- No metabolism information (ADME/TK)
- No chronic exposure data are available
- No immunotoxicity, neurotoxicity, or carcinogenicity data are available

Challenges/Key Issues:

- Uncertainty around *in vivo* endocrine activity potential
- How to handle the testing program for the derivatives
- Predictions for target organ toxicity and hematological, neurotoxic, and carcinogenic concerns
- Prevalence of bisphenols - impacts on study design & interpretation
- How much exposure to the active chemical - ADME/TK profiles of BPS and derivatives
- Route of exposure (dietary or gavage)
- Critical window of exposure
- Comparing and contrasting bisphenol analogues

Specific Aims

- Characterize the dose-response effects of BPS on target organ systems with a focus on reproductive, developmental, neurological, and hematological endpoints
- Assess *in vivo* ADME/TK profiles for BPS and *in vitro* clearance and metabolism for BPS and BPS derivatives
- Determine the need for chronic toxicology studies
- Compare and contrast BPS *in vivo* and *in vitro* data with other analogues and derivatives to build a knowledge base of bisphenol chemicals

Proposed Approach:

Phase 1:

- Leverage initial NTP efforts:
  - Evaluation of *in vitro* and HTS data on bisphenol analogues for similarity profiling and endocrine activity
  - Include additional *in vitro* assays and alternative model testing
- ADME/TK characterization in rodents:
  - Oral and intravenous exposure of BPS and metabolism characterization of BPS derivatives
- *In vivo* toxicity evaluation of BPS in rodents:
  - Perinatal oral exposure dose range-finding study in rats
  - Short-term adult oral exposure toxicity study in mice

Proposed Approach:

Phase 2:

In vivo toxicity evaluation of BPS in rodents:

- Subchronic oral including perinatal exposure window to assess potential for reproductive toxicity, teratogenicity, and neurotoxicity in rats
- Adult oral exposure 90-day toxicity study in mice

Phase 3:

- Additional studies, as needed (e.g., carcinogenicity, immunotoxicity)
- Utilizing *in vitro* and *in vivo* data to compare and contrast select analogues

Significance and Expected Outcome

- Bisphenol S - focus on designing studies to evaluate BPS and derivatives:
  - Evaluation of potential endocrine activity of BPS and derivatives
  - *In vitro* and ADME/TK - comparison between BPS and derivatives
  - *In vivo* information - risk assessment of BPS and evaluation of human exposure and observed toxicities in a rodent model

Significance and Expected Outcome

- Prevalent nature and high exposure potential of bisphenols suggest that a more global understanding of the effects would be beneficial:
  - Endocrine activity and potential toxicities
  - Mechanisms of action
  - Comparing and contrasting analogue structural and biological similarities, include additional in vitro and alternative testing models for evaluations
  - Comparison of *in vitro* versus *in vivo* versus HTS versus alternative model results

Review questions:

1. Comment on the merit of the proposed project relative to the mission and goals of the NTP. The NTP's stated goals are to: Provide information on potentially hazardous substances to all stakeholders; Develop and validate improved testing methods; Strengthen the science base in toxicology; Coordinate toxicology testing programs across DHHS (http://ntp.niehs.nih.gov/go/about).

2. Comment on the clarity and validity of the rationale for the proposed project. Has the scope of the problem been adequately defined? Are the relevant knowledge gaps identified and clearly articulated?

3. Comment on the strategy and approach proposed to meet the stated objectives of the project. Are specific aims reasonable and clearly articulated? Is the scope of work proposed appropriate relative to the public health importance of the issue(s) under consideration? If not, what modifications do you recommend? Where steps to further refine the strategy and/or approach are proposed, are they appropriate?

4. There are challenges inherent to achieving the aims of any proposed project. Are the relevant challenges and/or key scientific issues identified and clearly articulated? Where approaches to overcome challenges are proposed, are they appropriate? Are you aware of other scientific issues that need to be considered?

5. Rate the overall significance and public health impact of this project as low, moderate, or high. Identify any elements of the proposed project that you feel are more important than others, and/or that have a higher likelihood of success at meeting pre-defined specific aims.

6. Provide any other comments you feel NTP staff should consider in developing this project.

Back- up slides

EPA - Screening Level Toxicity Hazard Summary for BPA Alternatives:

	Human Health											Aquatic Toxicity		Environmental Fate	
Chemical	Acute Toxicity	Carcinogenicity	Genotoxicity	Reproductive	Developmental	Neurological	Repeated Dose	Skin Sensitization	Respiratory Sensitization	Eye Irritant	Dermal Irritant	Acute	Chronic	Persistence	Bioaccumulation
BPA	L	M	L	M	H	*M*	M	M		M	M	H	H	VL	L
BPS	L	*M*	M	M	M	*M*	H	L		L	L	M	M	M	L
2,4-BPS…	*L**	*M*	M	*M**	*M**	*M*	*H**	*L**		*L**	*L**	*M*	*H*	*M*	*L*
TGSA…	L	*M*	L	*M**	*M**	*M*	H	M	*M*	L	VL	H	M	*H*	*L*
BPS-MAE…	L	*M**	M	*M**	*M**	*M*	L	L	*M*	L	VL	H	H	*H*	*L*
BPS-MPE…	L	*M*	*M**	*M**	*M**	*M*	*H**	L		L	L	VH	*H*	*H*	*M*
D-8…	L	*M*	L	*M**	*M**	*M*	M	*L**		L*	L*	H	*H*	M	M
D-90….	L	*M*	L	*L*	*L*	*M*	*M*	L		M	VL	*L**	*L**	*VH*	*H*

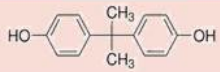
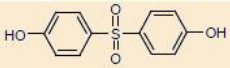
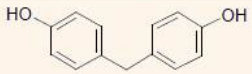
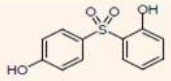
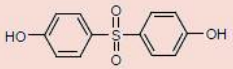
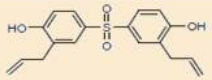
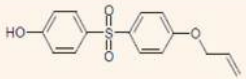
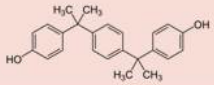
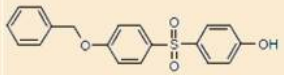
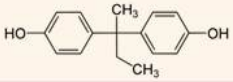
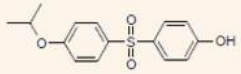
This table only contains information regarding the inherent hazards of the chemicals evaluated. Evaluation of risk considers both the hazard and exposure. **VL = Very Low hazard, L = Low hazard, M = Moderate hazard, H = High hazard, VH = Very High hazard . Endpoints in colored text were assigned based on empirical data. Endpoints in black italics were assigned using values from estimation software and professional judgment.**

* Based on analogy to experimental data for a structurally similar compound.

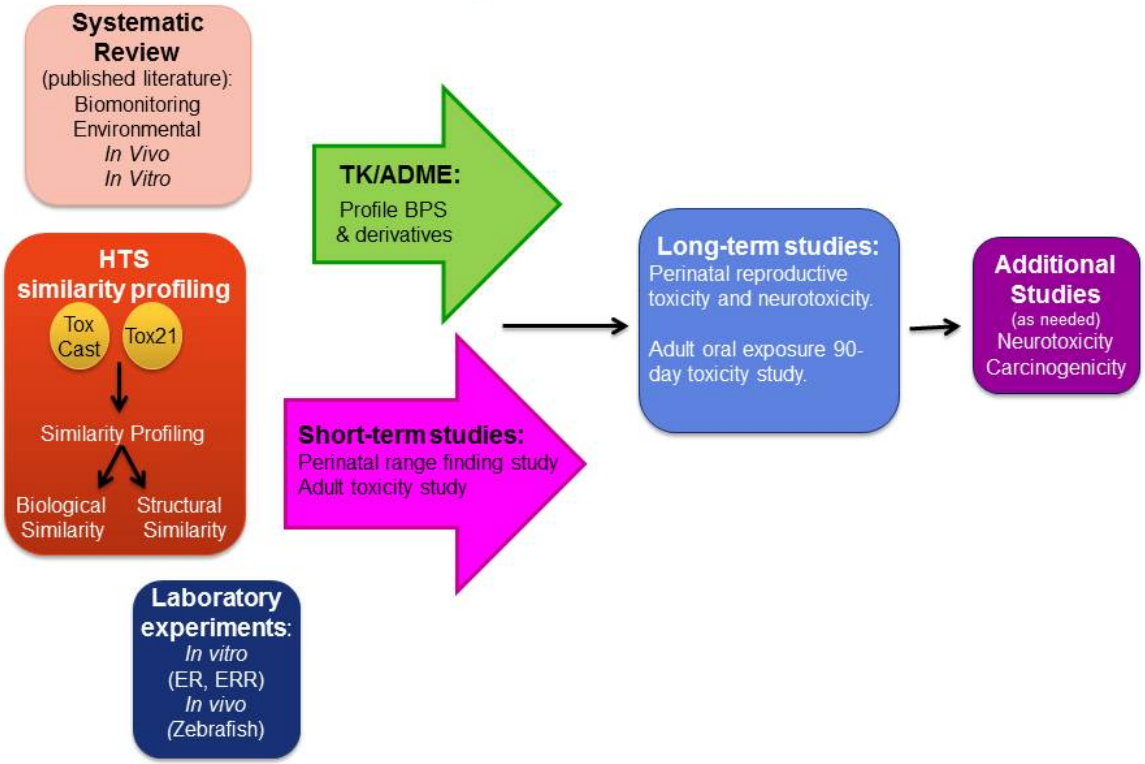
Bisphenol Analogs and BPS Derivatives:

BPA Analogues – structurally similar chemicals.

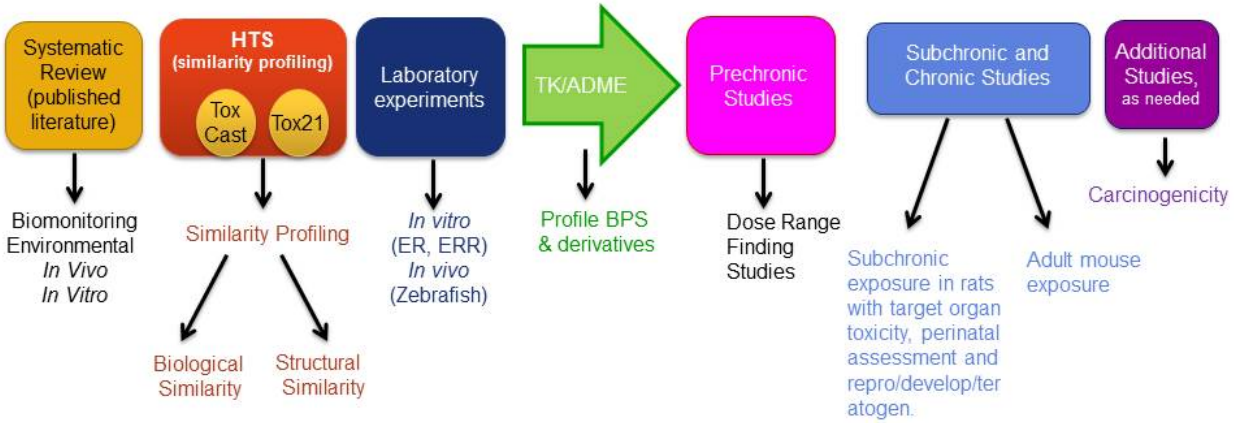
BPS Derivatives – same core structure as BPS.

Example Bisphenol Analogues	Analogue Structures	Example BPS Derivatives	Derivative Structures
BPA		BPS	
BPF		2,4-BPS 2,4'-Bis(hydroxyphenyl) sulfone	
BPS		TGSA Bis-(3-allyl-4-hydroxyphenyl) sulfone	
BPAF		BPS-MAE Phenol,4-[[4-(2-propen-1-yloxy)phenyl] sulfonyl]	
BPP		BPS-MPE 4-Hydroxy-4'-benzyloxydiphenylsulfone	
BPB		D-8 4-Hydroxyphenyl 4-isoprooxyphenylsulfone	
		D-90 4-[4'-[(1'-methylethyloxy) phenyl]sulfonyl]phenol	

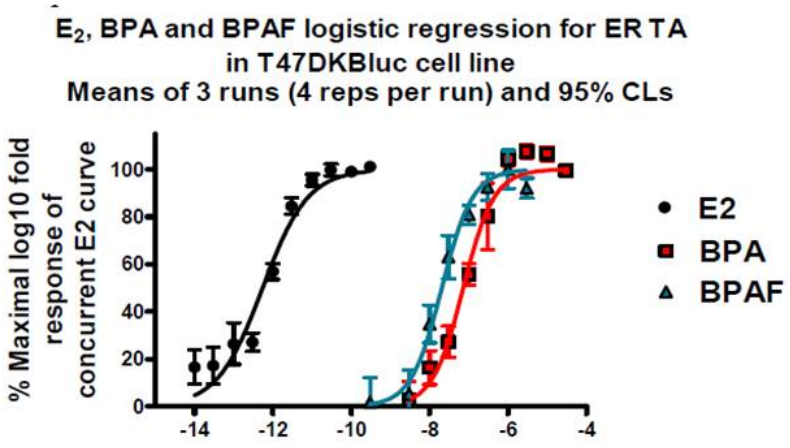
Proposed Plan:



Proposed Plan:



BPA vs BPAF Uterotrophic Assay:

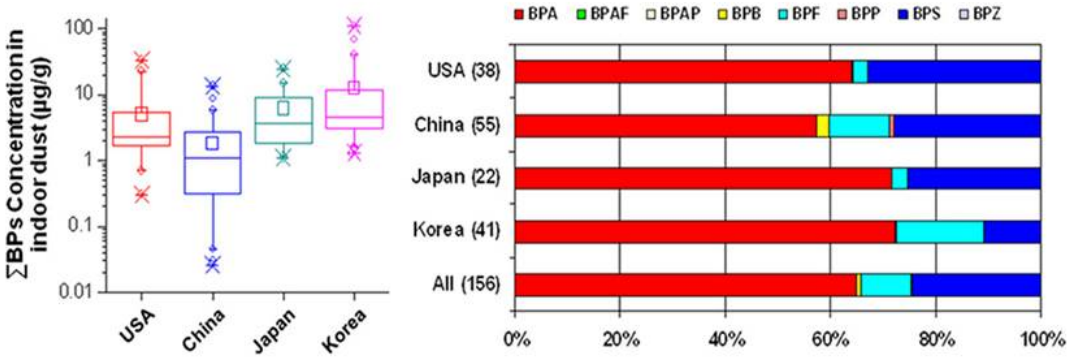


	E2	BPA	BPAF
EC50	5.471e-013	7.226e-008	2.045e-008

$p < 0.001$ for all comparisons

Toxicol Sci. 2010 Aug;116(2):477-87

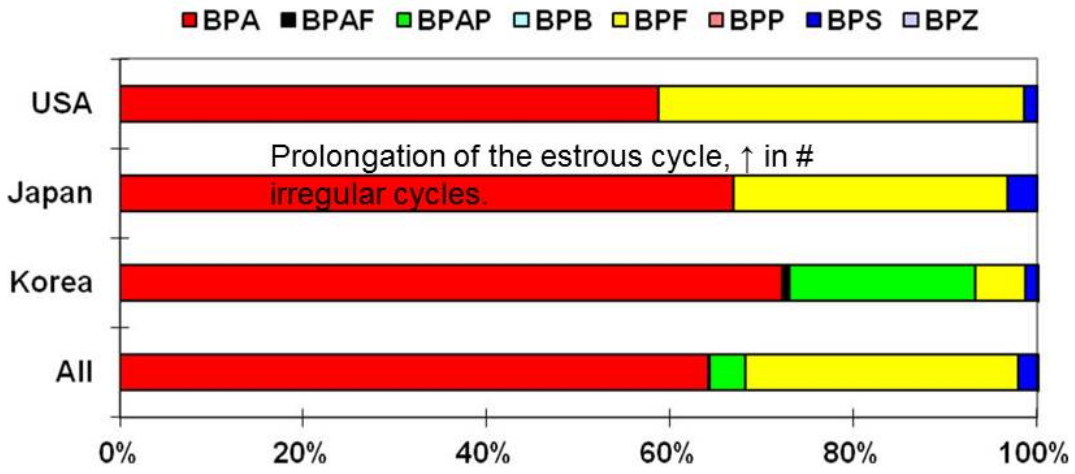
Bisphenol Analogues in Indoor Dust



Liao, C., et al., *Occurrence of eight bisphenol analogues in indoor dust from the United States and several Asian countries: implications for human exposure.* Environ Sci Technol., 2012. **46**(16): p. 9138-45.

Bisphenol Analogues in Sediment

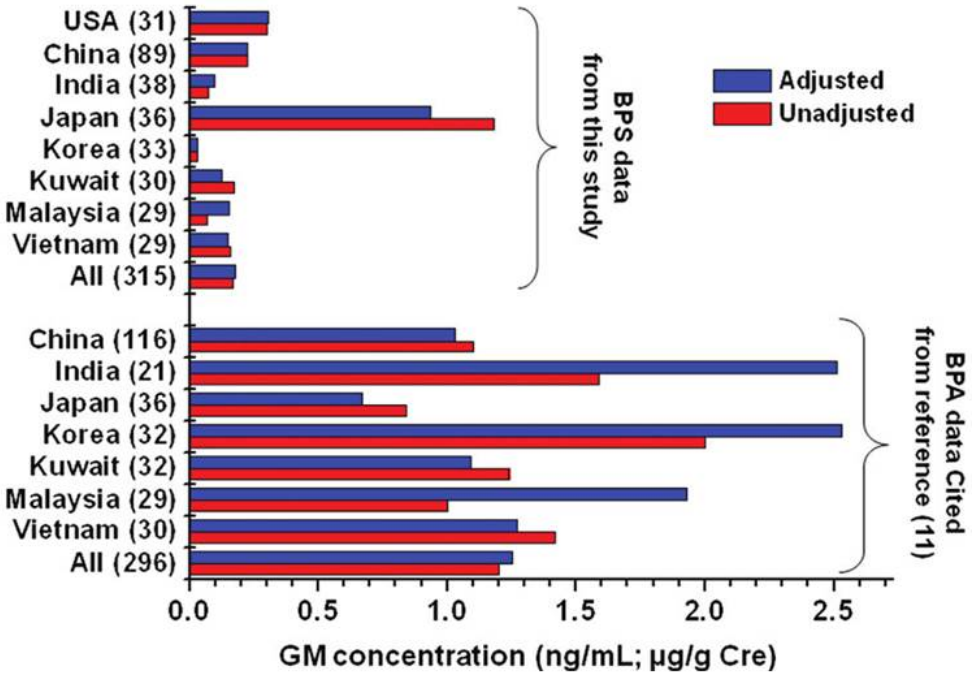
Katie Pelch



Liao, C., et al., *Bisphenol analogues in sediments from industrialized areas in the United States, Japan, and Korea: spatial and temporal distributions.* Environ Sci Technol., 2012. **46**(21): p. 11558-65.

BPS is Detected in Urine

Katie Pelch



Liao, C., et al., *Bisphenol S in urine from the United States and seven Asian countries: occurrence and human exposures.* Environ Sci Technol., 2012. **46**(12): p. 6860-6.

Literature Review Summary of BPS *in vitro* data:

Katie Pelch

Reference	Endpoint	Cell Line	Dose Range (μM)	Assay Type
Masuno, 2005	adipocyte differentiation	3T3-L1	86	gene expression
Masuno, 2005	adipocyte differentiation	3T3-L1	86	reporter gene
Molina-Molina, 2013	androgenic activity	MCF-7 AR1	0.01-10	A-screen
Teng, 2013	androgenic activity	CV1	0.01-100	reporter gene
Molina-Molina, 2013	androgenic activity	PALM	0.01-10	reporter gene
Teng, 2013	anti-androgenic activity	CV1		reporter gene
Kitamura, 2005	anti-androgenic activity	NIH3T3		reporter gene
Molina-Molina, 2013	anti-androgenic activity	PALM	0.01-10	reporter gene
Teng, 2013	anti-estrogenic activity	CV1		reporter gene
Vinas, 2013	apoptosis	GH3/B6/F10	0.00000001-0.01	activation of caspases
Vinas, 2013	apoptosis	GH3/B6/F10	0.00000001-0.01	activation of caspases
Lee, 2013	Cell viability	DT40	15.625-250	ATP assay
Lee, 2013	chromosomal aberration	DT40 RAD54-/-	62.5-250	chromosomal aberration
Hashimoto, 2000	ER binding		0.1-1000	Fluorescence polarization
Hashimoto, 2001	ER binding		0.1-1000	Fluorescence polarization
Laws, 2006	ER binding		10-100	relative binding
Molina-Molina, 2013	ER binding	HELN-hERα	0.01-10	whole cell competitive binding
Molina-Molina, 2013	ER binding	HELN-hERβ	0.01-10	whole cell competitive binding
Vinas, 2013	estrogenic activity	GH3/B6/F10	0.000000001-0.1	cell proliferation
Hashimoto, 2000	estrogenic activity	MCF-7	0.1-1000	E-screen
Hashimoto, 2001	estrogenic activity	MCF-7	0.001-100	E-screen
Molina-Molina, 2013	estrogenic activity	MCF-7	0.01-10	E-screen
Grignard, 2012	estrogenic activity	BG1Luc 4E2		reporter gene
Teng, 2013	estrogenic activity	CV1	0.001-50	reporter gene
Vinas, 2013	estrogenic activity	GH3/B6/F10	0.000000001-0.1	reporter gene
Molina-Molina, 2013	estrogenic activity	HELN-hERα	0.01-10	reporter gene
Molina-Molina, 2013	estrogenic activity	HELN-hERβ	0.01-10	reporter gene
Kitamura, 2005	estrogenic activity	MCF-7		reporter gene
Kuruto-Niwa, 2005	estrogenic activity	MCF-7	0.1-100	reporter gene
Grignard, 2012	estrogenic activity	MELN		reporter gene
Molina-Molina, 2013	estrogenic activity	MELN	0.01-10	reporter gene
Nishihara, 2000	estrogenic activity	[S. cerevisiae]		Yeast two-hybrid
Hashimoto, 2000	estrogenic activity	[S. cerevisiae]	0.1-1000	Yeast two-hybrid
Hashimoto, 2001	estrogenic activity	S. cerevisiae Y190	0.1-1000	Yeast two-hybrid
Hashimoto, 2001	estrogenic activity	S. cerevisiae Y190	0.1-1000	Yeast two-hybrid
Chen, 2002	estrogenic activity	S. cerevisiae Y190	[0.04-800]	Yeast two-hybrid
Vinas, 2013	non-genomic signaling	GH3/B6/F10	0.000000001-0.1	pERK response
Vinas, 2013	non-genomic signaling	GH3/B6/F10	0.000000001-0.1	pJNK response
Molina-Molina, 2013	pregnane X activity	HG5LN-PXR	0.01-10	reporter gene
Sui, 2012	pregnane X activity	HepG2	5-20	reporter gene (human)
Sui, 2012	pregnane X activity	HepG2	5-20	reporter gene (mouse)
Nishigori, 2012	RORγ binding		0.00001-10	competitive binding